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Substitute Form PTO 1390 U.S. Department of Commerce Patent and Trademark Office	Attorney's Docket Number: 50165/013001
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371	U.S. Application Number:

INTERNATIONAL APPLICATION NUMBER	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/IE00/00099	August 14, 2000	August 18, 1999

TITLE OF INVENTION:	PROCESS TO MAKE A SUSTAINED RELEASE FORMULATION
APPLICANTS FOR DO/EO/US:	Thomas Ciarán Loughman

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1.	■ This is a FIRST submission of items concerning a filing under 35 U.S.C. § 371.
2.	□ This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. § 371.
3.	■ This is an express request to begin national examination procedures (35 U.S.C. § 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. § 371(b) and PCT Articles 22 and 39(1).
4.	■ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5.	A copy of the International Application as filed (35 U.S.C. § 371(c)(2)). ■ a. is transmitted herewith (required only if not transmitted by the International Bureau). □ b. has been transmitted by the International Bureau. □ c. Is not required, as the application was filed with the United States Receiving Office (RO/US).
6.	□ A translation of the International Application into English (35 U.S.C. § 371(c)(2).
7.	Amendments to the claims of the International Application under PCT Article 19 (35 U.S C. § 371(c)(3)). □ a. are transmitted herewith (required only if not transmitted by the International Bureau). □ b. have been transmitted by the International Bureau. □ c. have not been made; however, the time limit for making such amendments has NOT expired. ■d. have not been made and will not be made.
8.	□ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. § 371(c)(3)).
9.	■ An (unsigned) oath or declaration of the inventors (35 U.S.C. § 371(c)(4)).
10.	□ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. §371 (c)(5).
11.	□ An Information Disclosure Statement under 37 C.F.R. §§ 1.97 and 1.98.
12.	□ An assignment for recording. A separate cover sheet in compliance with 37 §§ 3.28 and 3.31 is included.
13.	□ A FIRST preliminary amendment. □ A SECOND or SUBSEQUENT preliminary amendment.
14.	□ A substitute specification.
15.	□ A change of power of attorney and/or address letter.
16.	□ Other items or information:

17.	■ The following fees are submitted: BASIC NATIONAL FEE (37 C.F.R. § 1.492(A)(1)-(5)): Neither international preliminary examination fee (37 C.F.R. § 1.482) nor international search fee (37 C.F.R. § 1.455(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO $ 1040.00 International preliminary examination fee (37 C.F.R. § 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO $ 890.00 International preliminary examination fee (37 C.F.R. § 1.482) not paid to USPTO but international search fee (37 C.F.R. § 1.445(a)(2)) paid to USPTO $740.00 International preliminary examination fee (37 C.F.R. § 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1) - (4) $ 710.00 International preliminary examination fee paid to USPTO (37 C.F.R. § 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) $ 100.00		$890.00	

ENTER APPROPRIATE BASIC FEE AMOUNT =				$890.00	
Surcharge of $130 for furnishing the oath or declaration later than □ 20 OR □ 30 months from the earliest claimed priority date (37 C.F.R. § 1.492(e)).				$	
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	[9] - 20 =	0	x $18	$	
Independent claims	[2] - 3 =	0	x $84	$	
Multiple dependent claims (if applicable)			+ $280		
TOTAL OF ABOVE CALCULATIONS =				$890.00	
Reduction of 1/2 for filing by small entity, if applicable. [**Applicant claims small entity status under 37 C.F.R. § 1.27**]				$	
SUBTOTAL =				$	
Processing fee of $130.00 for furnishing the English translation later than □ 20 OR □ 30 months from the earliest claimed priority date (37 C.F.R. § 1.492(f)). +				$	
TOTAL NATIONAL FEE =				$	
Fee for recording the enclosed assignment (37 C.F.R. 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 C.F.R. §§ 3.28, 3.31). $40.00 per property. +				$	
TOTAL FEES ENCLOSED =				$ 890.00	
				Amount to be refunded	$
				charged	$

■1,170 a. A check in the amount of $890.00 to cover the above fees is enclosed.
□ b. Please charge my Deposit Account No. 03-2095 in the amount of $ [**.**] to cover the above fees.
■ c. The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment, to Deposit Account No. 03-2095.

NOTE: Where an appropriate time limit under 37 C.F.R. §§ 1.494 or 1.495 has not been met, a petition to revive (37 C.F.R. § 1.137(a) or (b) must be filed and granted to restore the application to pending status.

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PATENT
ATTORNEY DOCKET NO. 50165/013001

Certificate of Mailing: Date of Deposit: July 8, 2002

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Loughman, T.C.	Art Unit:	Not Assigned Yet
Serial No.:	10/049,692	Examiner:	Not Assigned Yet
Filed:	February 15, 2002	Customer No.:	21559
Title:	PROCESS TO MAKE A SUSTAINED RELEASE FORMULATION		

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Washington, D.C. 20231

PRELIMINARY AMENDMENT

Prior to examination, kindly amend the above-referenced application as follows:

In the Claims:

Please cancel claims 8 and 9 without prejudice.

REMARKS

If there are any charges or credits not covered, please apply them to Deposit Account No. 03-2095.

Respectfully submitted,

Date: July 8, 2007

for Paul T. Clark (Reg No. 42,506)
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21559
PATENT TRADEMARK OFFICE

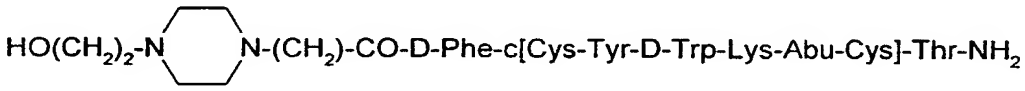
[redacted]

Description

Process to make a sustained release formulation

Technical Field

This invention pertains to a process for making a sustained release complex, Compound (I), which comprises Compound (A), having the formula



and a copolymer comprising poly-(l)-lactic-glycolic-tartaric acid (P(l)LGT), wherein the amino group of Compound (A) is ionically bound to a carboxyl group of the P(l)LGT.

Background Art

Many drug delivery systems have been developed, tested and utilized for the controlled *in vivo* release of pharmaceutical compositions. For example, polyesters such as poly(DL-lactic acid), poly(glycolic acid), poly(ε-caprolactone) and various other copolymers have been used to release biologically active molecules such as progesterone; these have been

in the form of microcapsules, films or rods (M. Chasin and R. Langer, editors, Biodegradable Polymers as Drug Delivery Systems, Dekker, NY 1990). Upon implantation of the polymer/therapeutic agent composition, for example, subcutaneously or intramuscularly, the therapeutic agent is released over a specific period of time. Such bio-compatible biodegradable polymeric systems are designed to permit the entrapped therapeutic agent to diffuse from the polymer matrix. Upon release of the therapeutic agent, the poller is degraded *in vivo*, obviating surgical removal of the implant. Although the factors that contribute to poller degradation are not well understood, it is believed that such degradation for polyesters may be regulated by the accessibility of ester linkages to non-enzymatic autocatalytic hydrolysis of the polymeric components.

Several EPO publications and U.S. Patents have addressed issues of polymer matrix design and its role in regulating the rate and extent of release of therapeutic agents *in vivo*.

For example, Deluca (EPO Publication 0 467 389 A2) describes a physical interaction between a hydrophobic biodegradable polymer and a protein or polypeptide. The composition formed was a mixture of a therapeutic agent and a hydrophobic polymer that sustained its diffusional release from the matrix after introduction into a subject.

Hutchinson (U.S. Pat. No. 4,767,628) controlled the release of a therapeutic agent by uniform dispersion in a polymeric device. It is disclosed that this formulation provides for controlled continuous release by the overlap of two phases: first, a diffusion-dependent leaching of the drug from the surface of the formulation; and second, releasing by aqueous channels induced by degradation of the polymer.

PCT publication WO 93/24150 discloses a sustained release formulation comprising a peptide having a basic group and a carboxy-terminated polyester.

US Patent No. 5,612,052 describes cation-exchanging microparticles made typically of carboxyl-bearing polyester chains onto which basic bioactive agents are immobilized to provide a control release system within an absorbable gel-forming liquid polyester.

Compound (A) is described and claimed in U.S. Patent No. 5,552,520.

The Applicant's PCT publication WO 97/40085 discloses biodegradable polyesters comprising lactic acid units, glycolic acid units and hydroxy-polycarboxylic acid units such as tartaric acid or pamoic acid and processes for making said polyesters. More specifically, it discloses

poly-lactide-glycolide-tartaric acid polymers in the ratio 65/33/2, respectively.

The Applicant's PCT publication WO 94/15587 discloses ionic conjugates of polyesters having free COOH groups with a bioactive peptide having at least one effective ionogenic amine. More specifically, it discloses that the polymers are made polycarboxylic by reacting the co-polymers with malic acid or citric acid. U.S. Patent No. 5,672,659, is the U.S. national phase continuation application of WO 94/15587. U.S. Patent No. 5,863,985 is a continuation of U.S. Patent No. 5,672,659. Pending U.S. Application No. 09/237,405 is a CIP of U.S. Patent No. 5,863,985, which additionally discloses a polyester which must include citric acid, ε-caprolactone and glycolide; compositions comprising the immediately foregoing polyesters and a polypeptide; a polyester that must include tartaric acid as one of its members; compositions comprising the immediately foregoing polyester and a polypeptide; and the foregoing compositions in the shape of rods which are optionally coated with a biodegradable polymer.

The Applicant's PCT publication WO 97/39738 discloses a method of making microparticles of a sustained release ionic conjugate as described in WO 94/15587.

The contents of the foregoing patents, applications and publications are incorporated herein in their entirety.

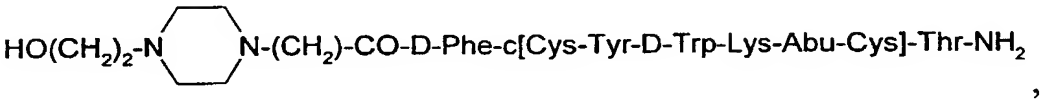
Disclosure of the Invention

The present invention is directed to a preferred process for making a preferred embodiment of a sustained release ionic conjugate of polymer and Compound (A), which is characterized by the resulting surprising and non-obvious property of zero-order release of Compound (A) from the conjugate. A further advantage of a process of the present invention is that the solvents used avoid side reactions and is less expensive to utilize than the process previously disclosed.

Brief description of the Drawing

Figure 1. Shows the *in vivo* release profile of Compound (A) from a sample of Compound (I) in dog, wherein the sample of Compound (I) consists of about 11.25% Compound (A), the polymer is l-lactide:glycolide:tartaric acid (72:27:1) and where Compound (I) is administered intramuscularly as microparticles. The irradiated sample refers to a sample of Compound (I) which was irradiated with γ-rays from a Cobalt source.

The present invention is directed to a process for making Compound (I), where Compound (I) comprises Compound (A),



(A)

and a polymer, wherein the polymer comprises lactide units, glycolide units and tartaric acid units where the ratio in the polymer of the lactide units is from and including 71% to 73%; of the glycolide units is from and including 26% to 28%; and of the tartaric acid units is from and including 1% to 3%; and where the amino group of Compound (A) is ionically bonded to a carboxylic group of the acid units of the polymer;

said process comprising the step of reacting an aqueous solution of Compound (A) with the polymer or a salt thereof, in a mixture of acetonitrile and water wherein the weight ratio of acetonitrile to water is about 3 to 1, respectively, at a temperature of about 0°C to 5°C until the formation of Compound (I) is substantially complete.

A preferred process of the immediately foregoing process is where the temperature of the reaction mixture is about 2.5°C; and the process comprises the additional step of isolating Compound (I).

In another aspect, the present invention comprises a process for making microparticles of Compound (I), as described hereinabove, said process comprising the steps of: nebulizing an ethyl acetate solution of Compound (I) into isopropyl alcohol to obtain a dispersion of microparticles of Compound (I),

wherein the concentration of Compound (I) in the ethyl acetate solution is about 8% to about 12% (W/W); the rate of spraying the solution of Compound (I) from the nebulizer into the isopropyl alcohol is about 4.9 ml/minute to about 5.1 ml/minute; the frequency setting of the nebulizer is such that the nebulizer does not spit the ethyl acetate solution of Compound (I); the volume of isopropyl alcohol is about 20 to 30 times volumetric excess compared to the ethyl acetate volume; and the temperature of isopropyl alcohol is about -60^{0}C to about -78^{0}C;

allowing the isopropyl alcohol to warm to about 0^{0}C to 22^{0}C; and isolating said microparticles from the isopropyl alcohol.

A preferred process of the immediately foregoing process is where the rate of spraying is about 5 ml/minute and the volume of isopropyl alcohol is about 20 times volumetric excess compared to the volume of ethyl acetate.

A preferred process of the immediately foregoing process is where the polymer comprises about 72% lactide units, about 27% glycolide units and about 1% tartaric acid units.

A preferred process of the immediately foregoing process is where the microparticles have a mean size of about 10 microns to about 100 microns.

A preferred process of the immediately foregoing process is where the microparticles have a mean size of about 40 microns to about 70 microns.

The term "about" as used herein in association with parameters or amounts, means that the parameter or amount is within ±5% of the stated parameter or amount.

The term "microparticle(s)" as used herein, refers to the micron size particles of the ionic conjugate comprising Compound (A) and poly-lactic-co-glycolic-co-L-(+)-tartaric acid polymer, which are preferably in essentially spherical form.

The instant application denotes amino acids using the standard three letter abbreviation known in the art, for example Phe = phenylalanine; Abu = α-aminobutyric acid.

General Procedures:

Co-Polymer formation: The co-polymer consisting of L-lactide, glycolide and L(+)-tartaric acid can be made according to methods well-known to those skilled in the art and as enabled herein. Accordingly, a reactor is loaded with monomers of glycolide, L-lactide and L(+)-tartaric acid and stannous 2-ethyl hexanoate in toluene solution. Preferably the molar percentages of L-lactide, glycolide, and L(+)-tartaric acid is about 72/27/1, respectively.

The L(+)-tartaric acid is previously dried preferably over silica gel in an Abderhalden drying apparatus for about 10 hours. The reactor is then put under vacuum with stirring to remove toluene. The reactor, under an atmosphere of oxygen-free nitrogen, is then heated, preferably immersed in an oil bath, temperature = about 180^{0}C to 190^{0}C, and stirring is increased to about 125 rpm. Prior to immersion, a heating tape (e.g., Thermolyne type 45500, input control setting = 4) is placed on the reactor lid. The time taken to completely melt the reactor contents is noted, typically about 15 minutes for a load of about 300g at about 180^{0}C. Samples are taken every hour during synthesis and analyzed by GPC to determine the percentage residual

monomer and to obtain values for average molecular weight by number (Mn) and by weight (Mw) distributions. Typical reaction times are of the order of about 9 to 15 hours. The final polymer is also analyzed by titration to determine an acid number in meq/g and by GC to determine residual unreacted monomer content. Further analyses include IR (detection of characteristic C=O peak); NMR (determination of lactide and glycolide content in polymer) and residual tin (determination of residual tin due to use of stannous 2-ethyl hexanoate as catalyst).

Purification/Sodium salt formation of the above copolymer: Residual monomer (typically <5% (W/W)) is removed and the copolymer is converted to it's sodium salt form (to promote ionic salt formation) in one step. The poly-L-lactic-co-glycolic-co-L(+)-tartaric acid copolymer (PLGTA) is dissolved in acetone by sonication in a sonication bath to give a solution with a concentration in the range of 19 - 21% PLGTA by weight.

To this solution is added a weak solution of an inorganic base such as NaOH or Na_2CO_3, preferably 0.2M sodium carbonate - Na_2CO_3 is used, in an amount so that the resulting concentration of sodium is 1 to 2 times molar excess, preferably 1.2 times molar excess, over copolymer carboxyl groups. The solution is left to stir for about 15 to 60 minutes, preferably 30 minutes, at room temp. to aid sodium salt formation. It is then fed at about 50 to 300ml/min, preferably about 100ml/min, into a jacketed reactor

containing de-ionized water cooled to about 1 to 4^{0}C, preferably 2.5^{0}C, using a circulation bath; the amount of water is about 20 to 30 times volumetric excess over acetone, preferably 20:1 volumetric excess over acetone. The water is stirred at a rate sufficient to create surface turbulence in order to avoid polymer agglomeration during precipitation using a paddle linked to a stirrer motor.

Once precipitation is complete, the dispersion is left to stir for a further 30 to 60 minutes to aid monomer removal before being placed in centrifuge bottles and spun down. The supernatant is discarded and the cakes are resuspended in further de-ionized water, re-spun and dried, preferably by lyophilization.

Preparation of a Compound (A) Polymer Ionic Conjugate: The synthesis entails binding Compound (A) to the copolymer sodium salt in a medium in which both are soluble, preferably 3:1 (W/W) acetonitrile:water, followed by precipitation of the resulting ionic conjugate in de-ionized water and recovery of the water-insoluble conjugate precipitate formed.

A solution of the acetate salt of Compound (A) in de-ionized water is added to a solution consisting of a washed Na salt of 12,000 MW 71/28/1 to 73/26/1 PLGTA in acetonitrile (Range 24 - 26% (W/W) solution) to which a weak base, preferably 0.5M Na_2CO_3, has been added so that it results in about a 1.05 molar excess of Na over the acetate content of the

Compound (A) acetate salt, and left to stir for about 5 minutes to provide an alkaline environment, preferably pH 8, to neutralize Compound (A)'s acetate group. Approximate weight ratio of acetonitrile:water = 3:1. Based on target loading required (usually about 8% to about 12%), the quantity of Compound (A) required is determined. From this the volume of aqueous sodium carbonate required to neutralize the acetate of Compound (A) is determined and finally the volume of water for Compound (A) dissolution is calculated based on a desired final acetonitrile:water (including sodium carbonate added) volumetric ratio of about 3:1.

The Compound (A)-copolymer solution is left to stir for about 10 to 15 mins. at about 0 to 5^0C, preferably 2.5^0C to facilitate ionic binding and discourage covalent binding (by use of low temperature) between the two components. The solution is then fed at a rate of about 50 to 300ml/min into about a 20-30 to 1 volumetric excess of de-ionized water over the volume of acetonitrile in the foregoing 3:1 acetonitrile-water solution, stirred at a rate sufficient to provide surface agitation and avoid agglomeration and cooled to about 1^0C to 4^0C, preferably 1.7^0C, in a jacketed reactor connected to a circulation bath.

When precipitation is complete the dispersion is left to stir for a further 30 to 60 minutes to aid removal of water-soluble Compound (A)-oligomer compounds (oligomers are those lower molecular weight fractions of PLGTA, which are undesirable since they are water soluble) before

being placed in centrifuge bottles and spun at about 5000 rpm for about 15 minutes in a centrifuge. The resultant centrifuge cakes are resuspended in de-ionized water and re-spun. They are then frozen and dried by lyophilization for 2 days and Compound (I) (Compound (A) ionically bound to PLGTA) is recovered. The loading is determined by HPLC analysis of the supernatant for unbound Compound (A) and nitrogen analysis (the Compound (A) nitrogen content is known and the polymer contains no nitrogen whatsoever). Extraction of Compound (A) from Compound (I) followed by HPLC analysis also allows determination of loading.

Compound (I) Nebulization: In order to provide a formulation well-suited for injection into a patient, Compound (I) is formulated into microspheres by dissolving it in ethyl acetate and using ultrasonic atomization (a.k.a. nebulization) to spray the solution into cold temperature, about -60°C to -78°C, ethanol, isopropanol or a mixture of hexane and isopropanol, preferably isopropanol, which results in the formation of microspheres of Compound (I) upon contact of the Compound (I) solution with the cold isopropanol. The Compound (I)/ethyl acetate solution can be sterilized by passing it through a 0.2μm filter.

Compound (I) is dissolved in ethyl acetate preferably by sonication/stirring to give about 8% to about 12% (W/W) solution, preferably 12%, depending on polymer molecular weight and Compound

(A) loading, both of which may alter solution viscosity. This is fed at about 4.90 ml/min. to 5.10 ml/min., preferably 5.00 ml/min. to an industrial atomizer or nebulizer (Power– about 70%, Amplitude– about 80%, Frequency– about 34 to 35kHz, preferably 34.50kHz; in general the nebulizer should be powerful enough to generate a frequency which can uniformly spray (without "spitting") the Compound (I)/ethyl acetate solutions from about 8% to about 12% (W/W) in concentration, such concentrations lead to the formation of solid microspheres and the frequency should be such that a mean particle size of between 40 and 70 microns is obtained, which will allow ease of injection through a 21-gauge or a 19-gauge needle) and nebulized into a volume of isopropanol (IPA) that is 20 to 30 times, preferably 20 times, volumetric excess compared to the ethyl acetate volume, cooled to about -60^{0}C to -78^{0}C, cooling can be achieved, (e.g., via a reactor jacket, addition of dry ice or insertion of a cooling coil) and stirred at least at 200rpm (to avoid microsphere agglomeration). De-ionised water at a temperature of about 6^{0}C is fed at preferably 1.5L/min to the nebulizer jacket to eliminate any local heating effects which can cause fouling of the nebulizer tip due to ethyl acetate evaporation. The solution nebulized evenly and an off-white particulate dispersion is seen to form in the IPA. This is allowed to thaw to about 0^{0}C to 22^{0}C over a period of about 30 mins. to 2 hrs before passing it through a 125µm sieve (to remove any large non-injectable droplets/particles) and on to a Whatman no.1 filter paper where it is vacuum-filtered. The filter cake is rinsed with further IPA and then vacuum dried.

The present invention is illustrated by the following example but is not limited by the details thereof.

Modes for Carrying Out the Invention

Example 1

Ionic Conjugate of P(l)LGTA (72/27/1) and Compound A

Step A: Synthesis of 300g of P(l)LG/tartaric acid copolymer (l-lactide:glycolide:tartaric acid = 72:27:1)

A reactor was loaded with monomers of glycolide (Purac Biochem, Netherlands, 68.71g), lactide (Purac Biochem, Netherlands, 227.53g) and L(+)-Tartaric acid (Riedel-de Haen, Seelze, Germany, article number 33801, 3.75g) and stannous 2-ethyl hexanoate (Sigma, St. Louis, Missouri, USA, article number S-3252) in toluene (Riedel-de Haen, Seelze, Germany) solution (0.0982M, 4.47ml). This corresponded to molar percentages of 71.81%; 26.82%; and 1.36% respectively of L-lactide, glycolide, and L(+)- tartaric acid.

The L(+)-tartaric acid was previously dried over silica gel (Riedel-de Haen, Seelze, Germany) in an Abderhalden drying apparatus for about 10 hours. The reactor (connected to a pump via a liquid nitrogen trap) was then put under vacuum (0.04 mbar) with stirring for about 50 minutes to

remove toluene. The reactor, under an atmosphere of oxygen-free nitrogen (BOC gases, Dublin, Ireland, moisture content of 8VPM), was then immersed in an oil bath (Temperature = ~180^{0}C) and stirring was increased to 125 rpm. Prior to immersion, a heating tape (Thermolyne type 45500, input control setting = 4) was placed on the reactor lid. The time taken to completely melt the reactor contents was noted, typically about 15 minutes for a load of 300g at about 180^{0}C. Samples were taken every hour during synthesis and analyzed by GPC to determine the percentage residual monomer and to obtain values for average molecular weight by number (Mn) and by weight (Mw) distributions. Typical reaction times were of the order of about 15 hours. The final polymer was also analyzed by titration to determine an acid number in meq/g and by GC to determine residual unreacted monomer content. Further analyses include IR (detection of characteristic C=O peak); NMR (determination of lactide and glycolide content in polymer) and residual tin (determination of residual tin due to use of stannous 2-ethyl hexanoate as catalyst).

Step B: Purification/Sodium salt formation with the above copolymer

Residual monomer (typically <5% (W/W)) was removed and the copolymer was converted to it's sodium salt form (to promote ionic salt formation) in one step. 81.05g of a 12,000g/mol 72/27/1 poly-L-lactic-co-glycolic-co-L(+)-tartaric acid copolymer (acid number by titration = 0.231meq/g) was dissolved in 324.24g of acetone (Riedel-de Haen, Seelze, Germany) by sonication in a sonication bath (Branson, Danbury,

Connecticut, USA) to give a solution with a concentration of 20.00% PLGTA by weight.

To this solution was added 56.17ml of 0.2M Na_2CO_3 (Aldrich, Gillingham, Dorset, UK), thus providing a 1.2 times molar excess of sodium over copolymer carboxyl groups. The solution was left to stir for about 30 minutes at room temp. to aid sodium salt formation. It was then fed at ~100ml/min into a 10L jacketed reactor containing 8.2L of de-ionized water (approximately a 20:1 volumetric excess over acetone cooled to about 2.5^{0}C). This water was stirred at a rate sufficient enough to create surface turbulence and avoid polymer agglomeration during precipitation using a paddle linked to a stirrer motor.

Once precipitation was complete, the dispersion was left to stir for a further 30 mins. to aid monomer removal before being placed in centrifuge bottles and spun at 5000rpm for about 15 minutes in a Sorvall centrifuge (DuPont Sorvall Products, Wilmington, Delaware, USA). The supernatant was discarded and the cakes were resuspended in further de-ionized water, respun and frozen in a freezer (-13^{0}C) overnight before being dried in a small-scale lyophilizer (Edwards, Crawley, West Sussex, UK) the next day. This lyophilizer contains no coolant system. After 5 days of lyophilization 65.37g of washed copolymer were recovered representing a yield of 80.65%.

Step C: Preparation of Compound (I)

A solution of 1.27g of the acetate salt of Compound (A) (Batch 97K-8501 from Kinerton Ltd., Dublin, Ireland, potency = 85.8% (potency refers to the percent free base peptide present in the peptide acetate salt); acetate = 10.87%) in 5.87g of de-ionized water was added to a solution consisting of 8.01g of a washed Na salt of 12,000 MW 72/27/1 PLGTA in 24.84g acetonitrile (Riedel de-Haen) (24.38% (W/W) solution to which 2.41ml of 0.5M Na_2CO_3 (this corresponds to a 1.05 excess of Na over the acetate content of Compound (A)-acetate salt) had been added and left to stir for about 5 minutes to provide an alkaline environment (pH 8) for neutralization of Compound (A)'s acetate groups. Approximate weight ratio of acetonitrile:water = 3:1. Based on target loading required, the quantity of Compound (A) required was determined. From this the volume of aqueous sodium carbonate required to neutralize the acetate of Compound (A) was determined and finally the volume of water for Compound (A) dissolution was calculated based on a desired final acetonitrile:water (including sodium carbonate added) volumetric ratio of 3:1.

The Compound (A)-copolymer solution was left to stir for about 15 mins. at about 2.5^{0}C to facilitate ionic and discourage covalent binding between the two. The solution was then fed at ~100ml/min into 630ml (approximately a 20:1 volumetric excess over acetonitrile) of de-ionized water stirred at 350 rpm (to provide surface agitation and avoid Compound

(A)-copolymer agglomeration) and cooled to about 1.7^{0}C in a 6L jacketed reactor connected to a circulation bath.

When precipitation was complete the dispersion was left to stir for a further 30 minutes to aid removal of water-soluble Compound (A)-oligomer compounds before being placed in centrifuge bottles and spun at 5000 rpm for about 15 minutes in a Sorvall centrifuge (DuPont Sorvall Products, Wilmington, Delaware, USA). The resultant centrifuge cakes were resuspended in de-ionized water and re-spun. They were then frozen and dried by lyophilization for 2 days. 8.30g of the title product were recovered representing a yield of 91.38%. The loading was determined by HPLC analysis of the supernatant for unbound Compound (A) and nitrogen analysis (the Compound (A) nitrogen content is known and the polymer contains no nitrogen whatsoever). Extraction of Compound (A) from Compound (I) followed by HPLC analysis also allows determination of loading, which for the this example was 11.25%.

Step D: Compound (I) Nebulization

Compound (I), 8.27g, from step C was dissolved in 60.77g of ethyl acetate by sonication/stirring (room temp.) to give a 12.00% (W/W) solution. This was fed at 5ml/min to an industrial atomizer/nebulizer (Martin Walter Powersonic Model MW400GSIP, available from Sodeva of France) set at Power = 70%, Amplitude = 80%, Frequency = 34.50kHz, and nebulized into 1.35L of isopropyl alcohol (IPA) (20 times volumetric

excess compared to ethyl acetate volume) cooled to about -74 ± 4^0C (cooling achieved via reactor jacket) and stirred at 200rpm (to avoid microsphere agglomeration) in a jacketed reactor. De-ionised water at a temperature of 6^0C was fed at 1.5L/min to the nebulizer jacket to eliminate any local heating effects which can cause fouling of the nebulizer tip due to ethyl acetate evaporation. The solution nebulized evenly and an off-white particulate dispersion was seen to form in the IPA. This was allowed to thaw to about 0^0C - 4^0C over a period of about 30 mins. to 2 hrs before passing it through a 125μm sieve (to remove any large non-injectable droplets/particles) and on to a Whatman no.1 filter paper where it was vacuum-filtered. The filter cake was rinsed with further IPA and then vacuum dried. 6.88g of injectable material was obtained representing a yield of 83.19%. The microparticles of Compound (I) had a mean particle size of about 54 microns.

The *in vivo* release of Compound (A) from microparticles of Compound (I) can be and were tested according to the following description. The *in vivo* study was designed to evaluate the *in vivo* release profile of Compound (A) following the intramuscular administration of microparticles of Compound (I) to male Beagle dogs by means of the pharmacokinetic profile of Compound (A) following its administration.

Pharmaceutical formulations of microparticles of Compound (I) were administered intramuscularly in the rear leg muscles. Following a single

intramuscular administration of the irradiated or non-irradiated prepared pharmaceutical forms containing microparticles of Compound (I) (amount of microparticles injected corresponded to 5 mg of Compound (A) based upon the determination that Compound (A) loading in Compound (I) was 11.23%) to groups of six dogs each per pharmaceutical form. The quantitation of Compound (A) in serum samples and the pharmacokinetic analysis is conducted as follows.

Blood collection from the dogs are carried out before injection (time 0), and 5, 15 and 30 min; 1, 2, 4, 8 and 12 hrs; 1, 2, 3 and 4 days; then twice per week over the first month (for example on Mondays and Thursdays); and finally once a week from the second month until completing the experiment, when the serum levels of Compound (A) were no longer detected. However, the real times of blood collection were recorded and used in the pharmacokinetic analysis. Blood samples (5 ml) at times 0 (before i.m. administration) and at 7, 21, 35, 56 and 84 days after the i.m. injection and blood samples (4 ml) for the rest of sampling times, were taken through the jugular or the cephalic veins at the prescribed times.

The samples were placed in two fractions: one about 2.5 ml or 3.5 ml in certain fixed time samplings, in tubes that contain 50 and 80 µl, respectively, of a solution of aprotinin (10 ml of Trasylol® 500000 KJU lyophilized and rediluted in 2 ml of p.p.i. water) and the other one about 1.5 ml in tubes that were allowed to stand.

After the red cells clot, the tubes were centrifuged for 20 min at 30000 r.p.m. at +4°C. Serum with aprotinin were removed and stored in two fractions at -20°C until the sample was analyzed for Compound (A).

The concentration of Compound (A) in the serum samples were analyzed by a radioimmunoassay method. Standard curves with blank dog plasma and Compound (A) standard solutions were prepared daily. In this method the limit of quantification for Compound (A) in dog serum samples is about 0.050 nanograms (ng)/ml. The areas under the curve (AUC) and the maximum serum concentration (C_{max}) were normalized by the dose supplied (dose administered to each of the animals expressed in μg/kg). The index of the absorption rate (C_{max}/AUC) were also calculated.

The results of the foregoing experiment are shown in Figure 1.

Compound (A) or a pharmaceutically-acceptable salt thereof, Compound (I) or microparticles of Compound (I) can be administered by oral, parenteral (e.g., intramuscular, intraperitoneal, intravenous or subcutaneous injection, or implant), nasal, vaginal, rectal, sublingual or topical routes of administration and can be formulated with pharmaceutically acceptable carriers to provide dosage forms appropriate for each route of administration.

Solid dosage forms for oral administration include capsules, tablets, pills, powders and granules. In such solid dosage forms, the active compound is admixed with at least one inert pharmaceutically acceptable carrier such as sucrose, lactose, or starch. Such dosage forms can also comprise, as is normal practice, additional substances other than such inert diluents, e.g., lubricating agents such as magnesium stearate. In the case of capsules, tablets and pills, the dosage forms may also comprise buffering agents. Tablets and pills can additionally be prepared with enteric coatings.

Liquid dosage forms for oral administration include pharmaceutically acceptable emulsions, solutions, suspensions, syrups, the elixirs containing inert diluents commonly used in the art, such as water. Besides such inert diluents, compositions can also include adjuvants, such as wetting agents, emulsifying and suspending agents, and sweetening, flavoring and perfuming agents.

Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, or emulsions. Examples of non-aqueous solvents or vehicles are propylene glycol, polyethylene glycol, vegetable oils, such as olive oil and corn oil, gelatin, and injectable organic esters such as ethyl oleate. Such dosage forms may also contain adjuvants such as preserving, wetting, emulsifying, and dispersing agents. They may be sterilized by, for example, filtration through a bacteria-retaining filter, by incorporating sterilizing agents into the compositions, by irradiating the

compositions, or by heating the compositions. They can also be manufactured in the form of sterile solid compositions which can be dissolved in sterile water, or some other sterile injectable medium immediately before use.

Compositions for rectal or vaginal administration are preferably suppositories which may contain, in addition to the active substance, excipients such as coca butter or a suppository wax.

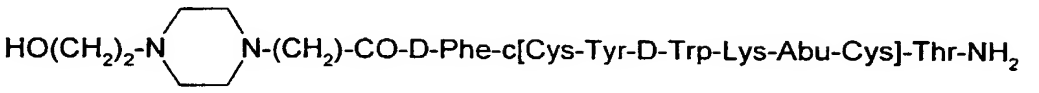
Compositions for nasal or sublingual administration are also prepared with standard excipients well known in the art.

It is preferred that the microparticles of Compound (I) be administered via parenteral administration or oral administration.

The effective dosage of the microparticles of Compound (I) to be administered to a patient can be determined by the attending physician or veterinarian and will be dependent upon the proper dosages contemplated for Compound (A) and the loading of Compound (A) in the microparticles of Compound (I). Such dosages will either be known or can be determined by one of ordinary skill in the art. Preferably the dosage should result in a level of at least 200 picograms/ml of Compound (A) in the patient.

Claims: -

1. A process for making Compound (I), where Compound (I) comprises Compound (A),



,

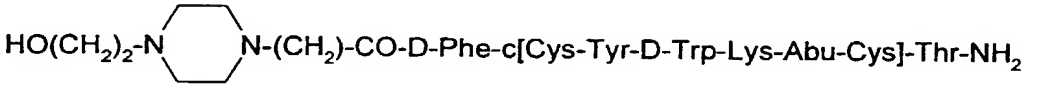
(A)

and a polymer, wherein the polymer comprises lactide units, glycolide units and tartaric acid units where the ratio in the polymer of the lactide units is from and including 71% to 73%; of the glycolide units is from and including 26% to 28%; and of the tartaric acid units is from and including 1% to 3%; and where the amino group of Compound (A) is ionically bonded to a carboxylic group of the acid units of the polymer;

said process comprising the step of reacting an aqueous solution of Compound (A) with the polymer or a salt thereof, in a mixture of acetonitrile and water wherein the weight ratio of acetonitrile to water is about 3 to 1, respectively, at a temperature of about 0°C to 5°C until the formation of Compound (I) is substantially complete.

2. A process according to claim 1 wherein, the temperature is about 2.5°C; and said process comprises the additional step of isolating Compound (I).

3. A process for making microparticles of Compound (I), where Compound (I) comprises Compound (A),



(A)

and a polymer, wherein the polymer comprises lactide units, glycolide units and tartaric acid units where the ratio in the polymer of the lactide units is from and including 71% to 73%; of the glycolide units is from and including 26% to 28%; and of the tartaric acid units is from and including 1% to 3%; and where the amino group of Compound (A) is ionically bonded to a carboxylic group of the acid units of the polymer;

said process comprising the steps of:

nebulizing an ethyl acetate solution of Compound (I) into isopropyl alcohol to obtain a dispersion of microparticles of Compound (I),

wherein the concentration of Compound (I) in the ethyl acetate solution is about 8% to about 12% (W/W); the rate of spraying the solution of Compound (I) from the nebulizer into the isopropyl alcohol is about 4.9 ml/minute to about 5.1 ml/minute; the frequency setting of the nebulizer is such that the nebulizer does not spit the ethyl acetate solution of Compound (I); the volume of isopropyl alcohol is about 20 to 30 times volumetric excess compared to the ethyl acetate volume; and the temperature of isopropyl alcohol is about -60^{0}C to about -78^{0}C;

allowing the isopropyl alcohol to warm to about 0^{0}C to 22^{0}C; and isolating said microparticles from the isopropyl alcohol.

4. A process according to claim 3, wherein the rate of spraying is about 5 ml/minute and the volume of isopropyl alcohol is about 20 times volumetric excess compared to the volume of ethyl acetate.

5. A process according to claim 4, wherein the polymer comprises about 72% lactide units, about 27% glycolide units and about 1% tartaric acid units.

6. A process according to claim 5, wherein the microparticles have a mean size of about 10 microns to about 100 microns.

7. A process according to claim 6, wherein the microparticles have a mean size of about 40 microns to about 70 microns.

8. A process according to claim 1 for making the Compound (I), substantially as hereinbefore described and exemplified.

9. A process according to claim 3 for making microparticles of Compound (I), substantially as hereinbefore described and exemplified.

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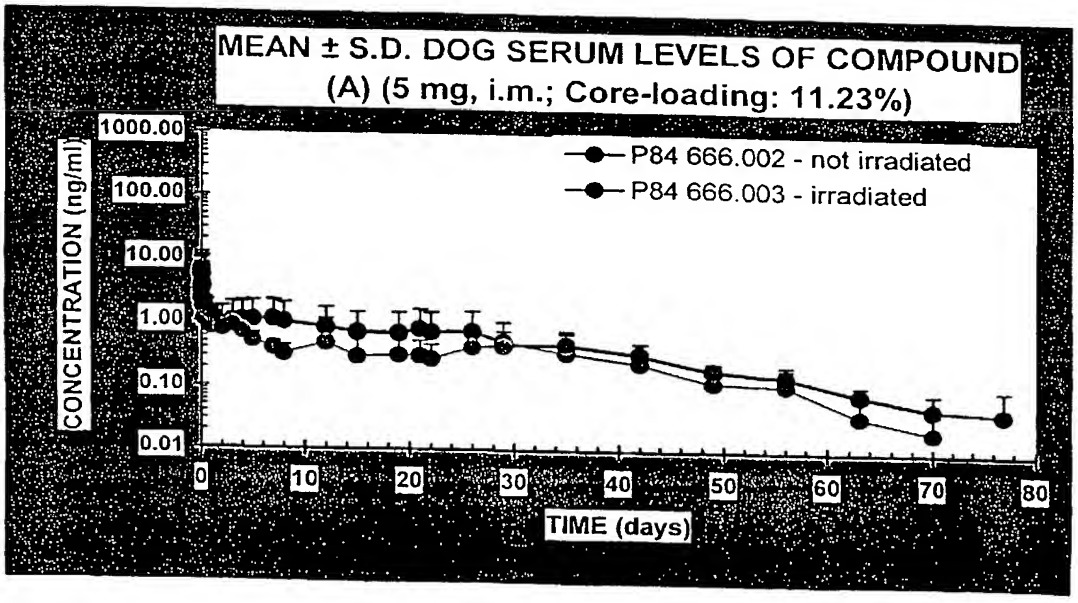
(54) Title: PROCESS TO MAKE A SUSTAINED RELEASE FORMULATION



(57) Abstract: This invention pertains to a process for making a sustained release complex. Compound (I), which comprises Compound (A), having formula (A), and a copolymer comprising poly-(1)-lactic-glycolic-tartaric acid (P(1)LGT), wherein the amino group of Compound (A) is ionically bound to a carboxyl group of the P(1)LGT.

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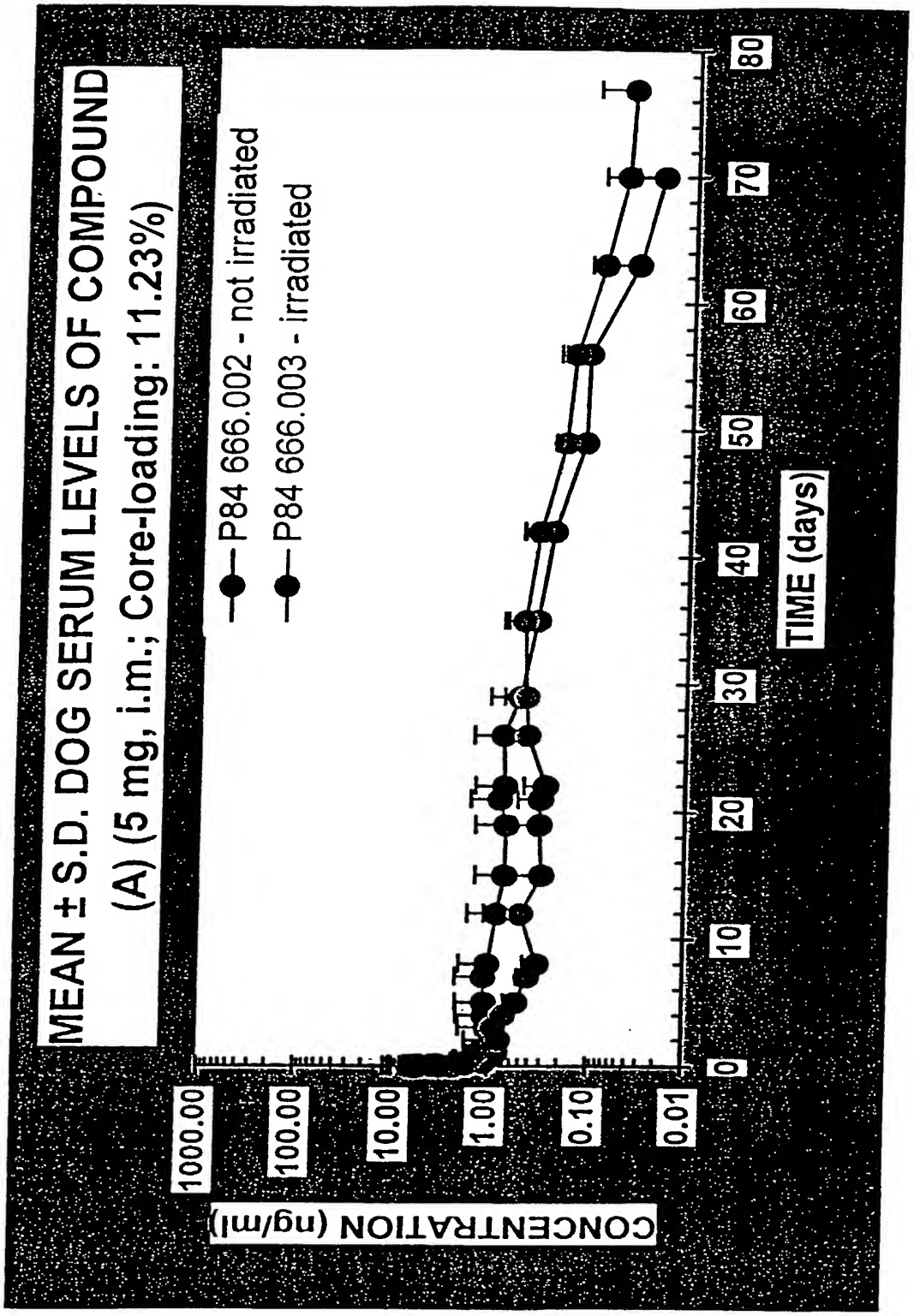
MEAN ± S.D. DOG SERUM LEVELS OF COMPOUND
(A) (5 mg, i.m.; Core-loading: 11.23%)
P84 666.002 - not irradiated
P84 666.003 - irradiated
CONCENTRATION (ng/ml)
1000.00
100.00
10.00
1.00
0.10
0.01
TIME (days)
0
10
20
30
40
50
60
70
80

Figure 1

PATENT
ATTORNEY DOCKET NO: 50165/013001

COMBINED DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled **PROCESS TO MAKE A SUSTAINED RELEASE FORMULATION**, the specification of which

☐ is attached hereto.
■ was filed on February 15, 2002 as Application Serial No. 10/049,692 .
☐ was described and claimed in PCT International Application No. _____
filed on _____ and as amended under PCT Article 19 on _____.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose all information I know to be material to patentability in accordance with Title 37, Code of Federal Regulations, § 1.56.

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PCT	PCT IE00/00099	August 14, 2000	Yes

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I hereby appoint the following attorneys and/or agents to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith: Paul T. Clark, Reg. No. 30,162, Karen L. Elbing, Ph.D. Reg. No. 35,238, Kristina Bieker-Brady, Ph.D. Reg. No. 39,109, Susan M. Michaud, Ph.D. Reg. No. 42,885, James D. DeCamp, Ph.D., Reg. No. 43,580, Sean J. Edman, Reg. No. 42,506, Timothy J. Douros, Reg. No. 41,716, Vicki Healy, Reg. No. 48,343, Alan F. Feeney, Reg. No. 43,609, and Brian R. Morrill, Reg. No. 42,908.

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